MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI

This application is a continuation-in-part of U.S. patent application Ser. No. 08/316,544, filed Sep. 30, 1994. The entire disclosure of that parent application is incorporated by reference herein.

FIELD OF THE INVENTION

The present invention is generally directed to the detection of genetic markers in a genomic system. The present invention is more specifically directed to the simultaneous amplification of multiple distinct polymorphic genetic loci using the polymerase chain reaction or other amplification systems to determine in one reaction the alleles of each locus contained within the multiplex system.

BACKGROUND OF THE INVENTION

In recent years, the discovery and development of polymorphic short tandem repeats (STRs) as genetic markers has stimulated progress in the development of linkage maps, the identification and characterization of diseased genes, and the simplification and precision of DNA typing.

Many loci, at least in the human genome, contain polymorphic STR regions (Adamson, D., et al. (1995) "A collection of ordered tetranucleotide-repeat markers from the human genome," *Am. J. Hum. Genet.* 57: 619–628; Murray, J. C., et al. (1994) "A comprehensive human linkage map with centimorgan density," *Science* 265: 2049–2054; Hudson, T. J., Engelstein, M., Lee, M. K., Ho, E. C., Rubenfield, M. J., Adams, C. P., Housman, D. E., and Dracopoli, N. C. (1992) "Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms," *Genomics* 13: 622–629). STR loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length. It is estimated that there are 2,000,000 expected trimeric and tetrameric STRs present as frequently as once every 15 kilobases (kb) in the human genome (Edwards et al. (1991) "DNA typing and genetic mapping with trimeric and tetrameric tandem repeats." *Am. J. Hum. Genet.* 49: 746–756; Beckman, J. S., and Weber, J. L. (1992) "Survey of human and rat microsatellites," *Genomics* 12: 627–631). Nearly half of the STR loci studied by Edwards et al. (1991) are polymorphic, which provides a rich source of genetic markers.

Variation in the number of short tandem repeat units at a particular locus causes the length of the DNA at that locus to vary from allele to allele and from individual to individual. Such length polymorphism is reminiscent of variable number of tandem repeats (VNTR) loci (Nakamura, Y., et al. (1987) "Variable number of tandem repeat (VNTR) markers for human gene mapping," *Science* 235: 1616–1622) and minisatellite loci (Jeffreys, A. J., et al. (1985) "Hypervariable 'minisatellite' regions in human DNA," *Nature* 314: 67–73), both of which contain considerably longer repeat units than STR loci. Such length polymorphism is also reminiscent of the dinucleotide repeat form of microsatellite loci (Litt, M. and Luty, J. A. (1989) "A hypervariable microsatellite revealed by in-vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene," *Am. J. Hum. Genet.* 44: 397–401, Tautz, D., et al. (1986) "Cryptic simplicity in DNA is a major source of genetic variation," *Nature* 322: 652–656, Weber, J. L. and May, P. E. (1989) "Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction," *Am. J. Hum. Genet.* 44: 388–396; Beckmann and Weber, (1992)), a form of microsatellite loci with shorter repeat units than STR loci.

Polymorphic STR loci are extremely useful markers for human identification, paternity testing and genetic mapping. STR loci may be amplified via the polymerase chain reaction (PCR) by employing specific primer sequences identified in the regions flanking the tandem repeat.

Alleles of these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another following electrophoretic separation by any suitable detection method including radioactivity, fluorescence, silver stain, and color.

To minimize labor, materials and analysis time, it is desirable to analyze multiple loci and/or more samples simultaneously. One approach for reaching this goal involves amplification of multiple loci simultaneously in a single reaction. Such "multiplex" amplifications, as they are called, have been described extensively in the literature. Multiplex amplification sets have been extensively developed for analysis of genes related to human genetic diseases such as Duchenne Muscular Dystrophy (Chamberlain, J. S., et al. (1988) "Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification," *Nucleic Acid Res.* 16: 11141–11156; Chamberlain, J. S., et al. (1989), "Multiple PCR for the diagnosis of Duchenne muscular dystrophy," In *PCR Protocols, A Guide to Methods and Application* (ed. Gelfand, D. H., et al.) pp. 272–281. Academic Press, San Diego, Calif.; Beggs, A. H., et al. (1990) "Detection of 98% DMD/BMD gene deletions by PCR," *Hum. Genet.* 86: 45–48; Clemens, P. R., et al. (1991). "Carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophy families, using dinucleotide repeat polymorphisms," *Am J. Hum. Genet.* 49: 951–960; Schwartz, J. S., et al. (1992) "Fluorescent multiple linkage analysis and carrier detection for Duchenne/Becker's muscular dystrophy," *Am J. Hum. Genet.* 51: 721–729; Covone, A. E., et al. (1992) "Screening Duchenne and Becker muscular dystrophy patients for deletions in 30 exons of the dystrophin gene by three-multiplex PCR," *Am. J. Hum. Genet.* 51: 675–677), Lesch-Nyhan Syndrome (Gibbs, R. A., et al. (1990) "Multiple DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families," *Genomics* 7: 235–244), Cystic Fibrosis (Estivill, X., et al. (1991) "Prenatal diagnosis of cystic fibrosis by multiplex PCR of mutation and microsatellite alleles," *Lancet* 338: 458; Fortina, P., et al. (1992) "Non-radioactive detection of the most common mutations in the cystic fibrosis transmembrane conductance regulator gene by multiplex polymerase chain reaction," *Hum. Genet.* 90: 375–378; Ferrie, R. M., et al. (1992) "Development, multiplexing, and application of ARMS tests for common mutations in the CFTR gene," *Am. J. Hum. Genet.* 51: 251–262; Morral, N. and Estivill, X. (1992) "Multiplex PCR amplification of three microsatellites within the CFTR gene," *Genomics* 51: 1362–1364), and Retinoblasma (Lohmann, D., et al. (1992) "Detection of small RB1 gene deletions in retinoblastoma by multiplex PCR and high-resolution gel electrophoresis," *Hum. Genet.* 89: 49–53). Multiplex amplification of polymorphic microsatellite markers (Clemens et al. (1991); Schwartz et al. (1992); Huang, T. H.-M., et al. (1992) "Genetic mapping of four dinucleotide repeat loci DXS435, DXS45, DXS454, DXS424, on the X chromosome using the multiplex polymerase chain reaction," *Genomics* 13: 375–380) and even STR markers (Edwards, A., et al. (1992) "Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups," *Genomics* 12: 241–253; Kimpton, C. P., et al.